ERITADENINES — NOVEL TYPE OF POTENT INHIBITORS OF S-ADENOSYL-L-HOMOCYSTEINE HYDROLASE*

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Rat liver SAH-hydrolase is strongly inhibited by four stereoisomeric 4-(adenin-9-yl)-2,3-dihydroxybutyric acids (eritadenines). D-Eritadenine, which is the most effective of the four, inactivates the catalytic activity of SAH-hydrolase at $IC_{50} = 1{\cdot}2 \cdot 10^{-8}$ mol l^{-1} in the hydrolytic reaction. The enzyme is irreversibly inhibited ($\tau/2 = 1{\cdot}6$ min). The inactivation activity decreases in the order D-*erythro*(2*R*,3*R*) > L-*erythro*(2*S*,3*S*) ≫ *threo*(2*S*,3*R*) > *threo*(2*R*,3*S*) configuration.

In the first communication of this series[1] we described the inhibitory effect of 9-((*S*)-2,3-dihydroxypropyl)adenine (*I*), an aliphatic nucleoside analogue with antiviral activity, on S-adenosyl-L-homocysteine hydrolase (SAH-hydrolase, EC 3.3.1.1). In view of this finding, we decided to investigate the interaction of SAH-hydrolase with other aliphatic adenine derivatives, containing hydroxy groups in the aliphatic chain. One of the most interesting groups of compounds of this series are the so-called eritadenines, bearing an ω-carboxy group in the aliphatic chain. In this communication we describe the character of the inhibitory effect of the four stereoisomeric 4-(adenin-9-yl)-2,3-dihydroxybutyric acids (eritadenines *II* – *V*) on rat liver SAH hydrolase. This enzyme which had been already used in our previous study was further purified by affinity chromatography on modified Sepharose 4B (ref.[2]) and was free of enzyme activities, degrading the substrates or products of the SAH-hydrolase catalyzed reaction[3]:

$$\text{adenosine} + \text{L-homocysteine} \underset{\text{hydrolase}}{\overset{\text{SAH-}}{\rightleftharpoons}} \text{S-adenosyl-L-homocysteine (SAH)}\,.$$

The inhibitory effect of eritadenines *II* – *V* on this enzyme was followed both in the direction of SAH synthesis and in the direction of its hydrolysis.

As seen from Fig. 1 and Table I, natural D-eritadenine *III* is the most potent of the four stereoisomers: a 50% enzyme inhibition is achieved at concentrations $0{\cdot}7 \cdot 10^{-8}$ mol l^{-1} and $7{\cdot}5 \cdot 10^{-8}$ mol l^{-1} for hydrolysis and synthesis, respectively. Under

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these conditions, the respective substrate-inhibitor ratios were 400 : 1 and 267 : 1. The L-*erythro*-enantiomer *II* (Fig. 1, Table I) exhibits somewhat lower activity whereas the *threo*-derivatives *IV* and *V* (Fig. 2, Table I) are 10 to 30 times less active than the *erythro*-isomers.

TABLE I

Effect of eritadenines upon reactions catalyzed by SAH-hydrolase from rat liver

Compound	Configuration	Synthesis[a]		Hydrolysis[b]	
		IC^1_{50}	IC^2_{50}	IC^3_{50}	IC^4_{50}
II	2S,3S	1·25	0·30	0·063	0·006
III	2R,3R	0·075	0·016	0·007	0·012
IV	2S,3R	12·5	10·0	1·50	0·60
V	2R,3S	35·0	20·0	2·0	1·00

[a] IC^1_{50}, IC^3_{50} designates concentrations (μmol l^{-1}) for 50% enzyme inhibition after 10 min incubation period without preincubation; [b] IC^2_{50}, IC^4_{50} concentrations (μmol l^{-1}) for 50% enzyme inactivation after 10 min preincubation period.

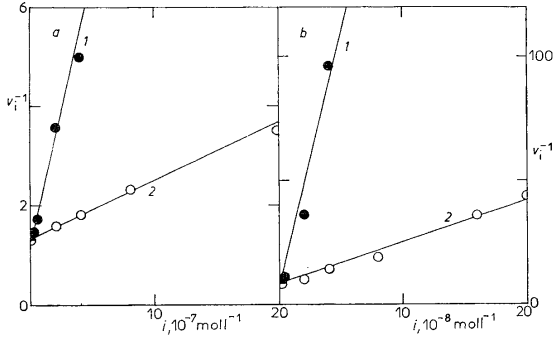


FIG. 1

Kinetics of SAH-hydrolase inhibition by compound *II* and *III* in the direction of synthesis (*a*) and hydrolysis (*b*). *1* compound *III*, *2* compound *II*. v_i [μmol min^{-1}] represents in *a* [SAH] . . min^{-1}, in *b* [Ino] . min^{-1}

TABLE II

Inactivation of rat liver SAH-hydrolase by eritadenines

Compound	Concentration 10^{-8} mol l^{-1}	$\tau/2$, min	
		synthesis	hydrolysis
II	**4**	—	**4**
	40	2·4	—
III	0·4	—	1·6
	2	—	2·0
	4	2·0	—
	12	1·5	—
IV	100	—	2·0
	250	3·0	—
	1 000	2·8	—
V	100	—	1·5
	250	6·0	—
	1 000	5·2	—

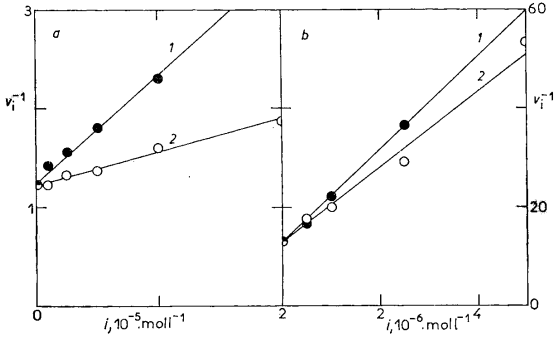


FIG. 2

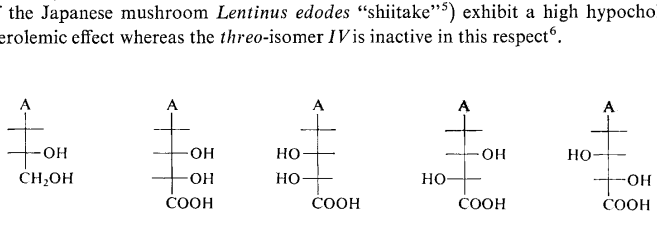
Kinetics of SAH-hydrolase inhibition by compound *IV* and *V* in the direction of synthesis (*a*) and hydrolysis (*b*). *1* compound *IV*, *2* compound *V*. v_i [μmol min^{-1}] represents in *a* [SAH] . . min^{-1}, in *b* [Ino] . min^{-1}

A more detailed study of the inhibitory activity revealed that the described inhibition of rat liver SAH-hydrolase by compounds *II* – *V* is irreversible. Their preincubation with the enzyme in the absence of L-homocysteine results in a very fast inactivation, its half-time (1·5 to 6 min) depending on structure and concentration of the eritadenines and on direction of the reaction (Table II). The presence of adenosine in the reaction mixture protects partially the enzyme; consequently, the synthetic reaction requires higher inhibitor concentration to achieve both 50% inhibition (IC^1_{50}) and 50% inactivation (IC^2_{50}) (Table I).

Time dependence of the SAH-hydrolase inactivation by compound *III* in the direction of SAH synthesis is given in Fig. 3. The enzyme is partially inactivated also by adenosine[4]; however, in our experiments $\tau/2$ of this reaction is 25 min at adenosine concentration $2 \cdot 10^{-5}$ mol l^{-1} (Fig. 3) whereas the compound *III* inactivates the enzyme already at a concentration 500 times lower ($\tau/2 = 1{\cdot}6$ min).

In spite of the fact that the inhibition of SAH-hydrolase by compound *I* is a reversible process we checked in this context also its effect of the enzyme inactivation under preincubation conditions. We found that in concentration $2 \cdot 10^{-5}$ mol l^{-1}. (comparable with that of adenosine), 9-((*S*)-2,3-dihydroxypropyl)adenine(*I*) exhibits only a negligible inactivation effect (Fig. 3).

Table I and Fig. 1 and 2 show that the inhibitory effect of the studied compounds decreases in the order *III* > *II* ≫ *IV* > *V*, both *erythro*-isomers being substantially more active than the *threo*-isomers. Inspection of models suggests that the *erythro*-derivatives *II* and *III* can adopt the zwitterionic structure in which conformation of the hydroxyls in positions 2 and 3 of the aliphatic chain is very similar to that in adenosine. On the other hand, such situation is not possible in the *threo*-isomers *IV* and *V*, or, alternatively, the distance between the carboxy and 6-amino groups increases; these conformational changes might cause a different affinity towards the enzyme at the binding site. It should be pointed out that both the *erythro*-isomers *II* and *III* (and particularly the natural D-eritadenine *III* which is the active compound of the Japanese mushroom *Lentinus edodes* "shiitake"[5]) exhibit a high hypocholesterolemic effect whereas the *threo*-isomer *IV* is inactive in this respect[6].



I *II* *III* *IV* *V*

In formulae *I* – *V*, A represents the adenin-9-yl residue.

Compounds *II* – *V* cannot be substrates for SAH-hydrolase; a direct experiment proved that their incubation with an SAH synthesizing or hydrolyzing system does not cause any observable changes of the inhibitors. Irreversible character of the enzyme inhibition obviously exludes an application of usual enzyme kinetics for characterization of their effect. A similar interaction with SAH-hydrolase was observed with 9-(β-D-arabinofuranosyl)adenine and 2'-deoxyadenosine[4]. The order of magnitude of the IC_{50} values for the compound *III* ($7 \cdot 10^{-9}$ mol l^{-1} to $7{\cdot}5 \cdot 10^{-8}$ mol l^{-1}; Table I) corresponds to the enzyme concentration (1·5 μg/ml) in the assay system (calculated for $2{\cdot}2 \cdot 10^5$ daltons of an enzyme from mammalian tissues[7,10]). We can thus assume that the affinity of D-eritadenine (*III*) to rat liver SAH-hydrolase is sufficiently high to inactivate the enzyme by an equimolar inhibitor concentration.

EXPERIMENTAL

Materials. D-Eritadenine (*III*) and L-eritadenine (*II*) were prepared by the described procedures[6,8] and freed from the accompanying impurities *via* methyl esters[9] to the HPLC homogeneity. The *threo*-isomers *IV* and *V* were synthesized according to ref.[8].

Enzyme preparation. SAH-hydrolase from rat liver was purified as described in our previous study[1] followed by an affinity chromatography step using modified AH-Sepharose 4B; *cf.*[2]. The enzyme preparation thus obtained possessed specific activity 0·60 I.U./mg protein.

Enzyme assays. The synthetic activity of SAH-hydrolase was determined (*cf.*[1]) in the incubation mixture (total volume, 0·25 ml) consisting of 80 mmol l^{-1} potassium phosphate pH 7·37, 2·4 mmol l^{-1} dithiothreitol, 3 mmol l^{-1} L-homocysteine, $2 \cdot 10^{-5}$ mol l^{-1} [^{14}C]-adenosine, 1·5 μg/ml enzyme protein, and appropriate amount of the inhibitor. The hydrolytic activity was estimated (*cf.*[1]) in the assay mixture (total volume 0·25 ml) consisting of 80 mmol l^{-1} potassium

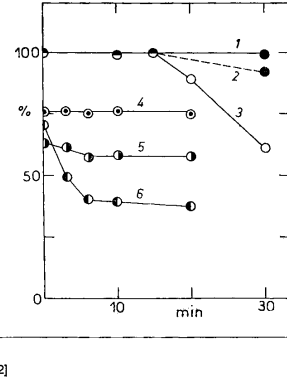


FIG. 3

Time course of inactivation of rat liver SAH-hydrolase in the synthetic direction. Preincubation of the SAH-hydrolase was carried out *1* in the absence of adenosine at 37°C, in the presence of $2{\cdot}2 \cdot 10^{-5}$ mol l^{-1} adenosine at 0°C, *3* $2 \cdot 10^{-5}$ mol l^{-1} at 37°C, *4* $0{\cdot}5 \cdot 10^{-5}$ mol l^{-1} 9-((*S*)-2,3-dihydroxypropyl)adenine (*I*) at 37°C, *5* $2 \cdot 10^{-5}$ mol . l^{-1} *I* at 37°C, *6* $4 \cdot 10^{-8}$ mol l^{-1} compound *III* at 37°C

phosphate pH 7·37, 0·1 mmol l^{-1} EDTA, 4 . 10^{-6} mol l^{-1} [^{14}C]-S-adenosyl-L-homocysteine, 1·5 μg/ml enzyme protein, and 1·6 I.U./ml adenosine aminohydrolase. The mixture was supplemented by inhibitors. The reactions were started by addition of the enzyme and the mixtures were incubated at 37°C for 10 min.

In the preincubation experiments, the enzyme was preincubated with inhibitors separately in 100 mmol l^{-1} potassium phosphate, pH 7·37, containing 1 mmol l^{-1} dithiothreitol and an aliquot of this mixture then supplemented with the rest of the reaction mixture to preserve original conditions required for the assay of synthesis or hydrolysis of SAH.

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